

B

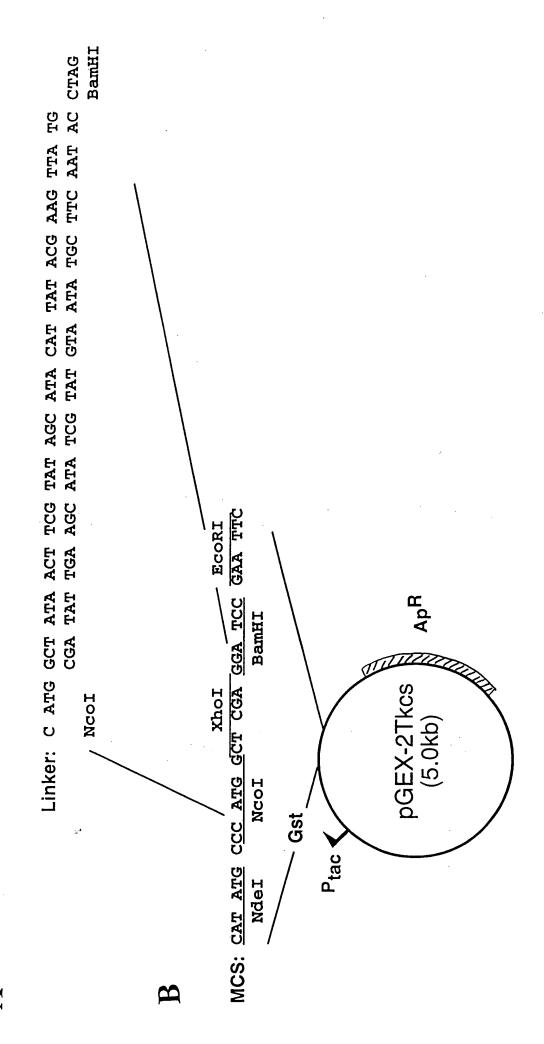
GC GGC CGC GGT ACC ATA ACT TCG TAT AGC ATA CAT TAT ACG AAG TTA TCT

EcoRI Smal Xhol Ndel Ncol BamHl Notl

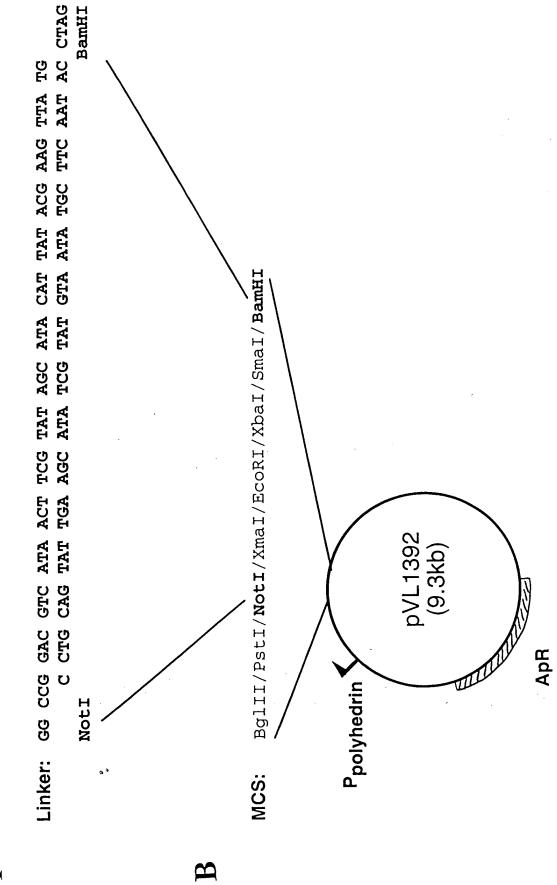
GGA ATT CCC CGG GCT CGA GAA CAT ATG GCC ATG GGG ATC CGC GGC CGC

HpaI Sall Sacl
AAT TGT TAA CAG ATC CGT CGA CGA GCT CGC TA (530)

Construction of pGst-lox:

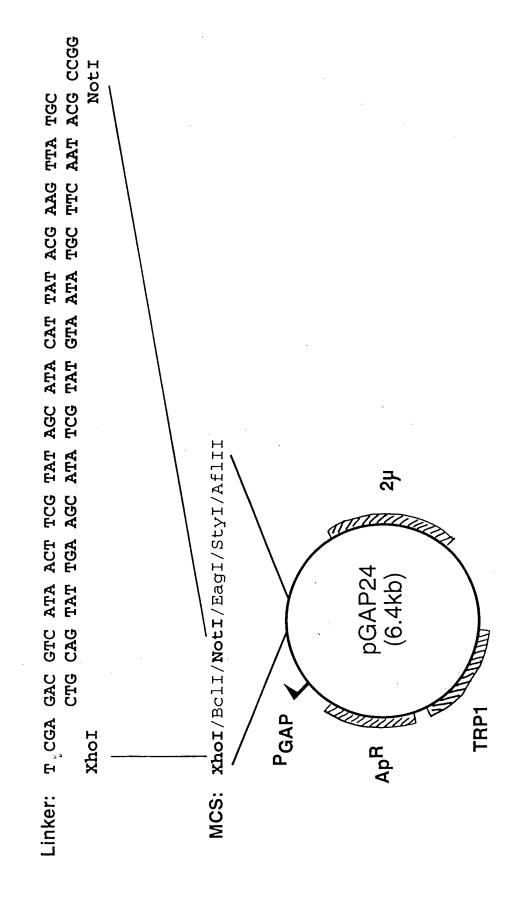


Construction of pVL1392-lox:



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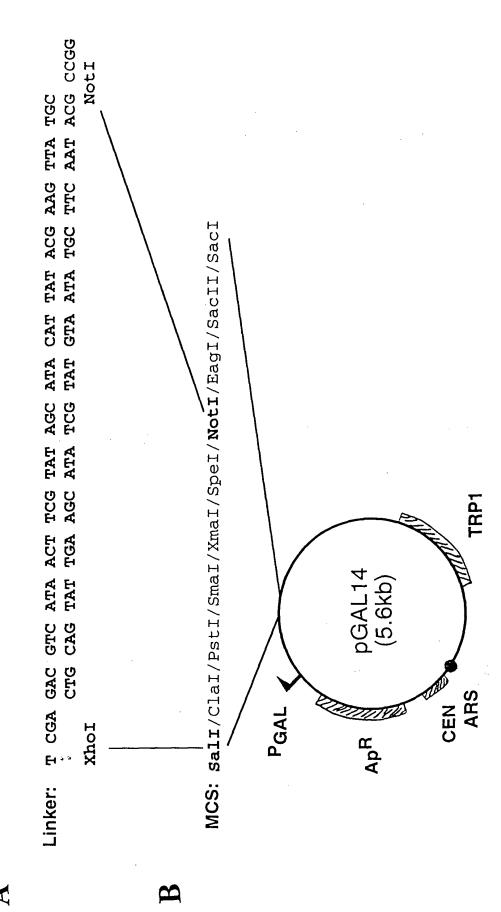
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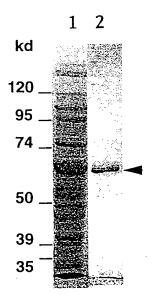


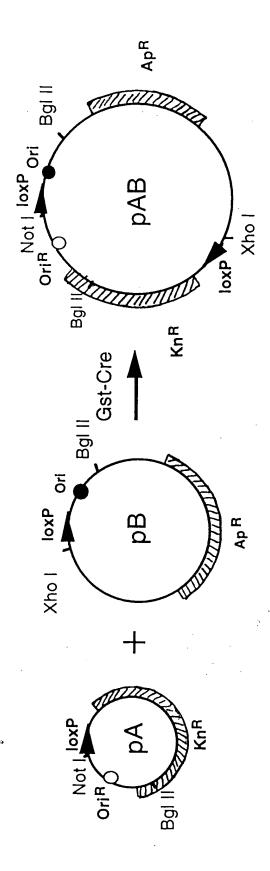
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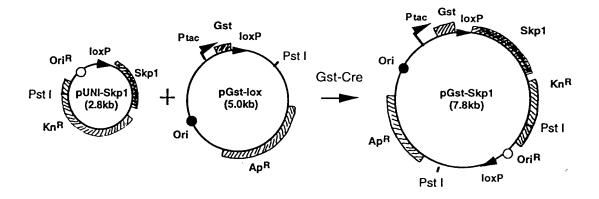
Construction of pGAL14-lox:



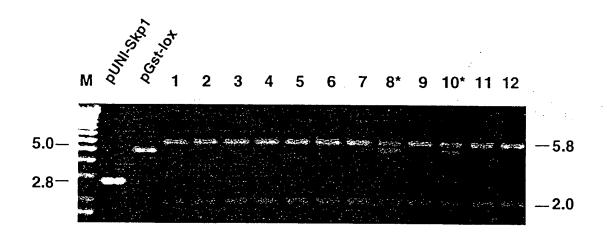




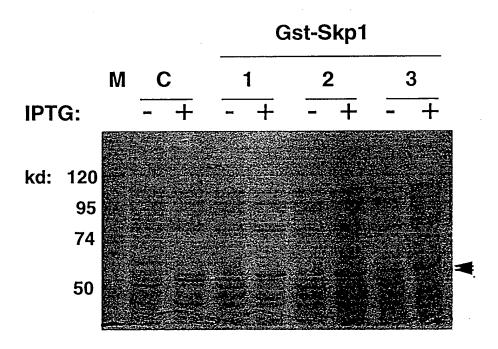
A



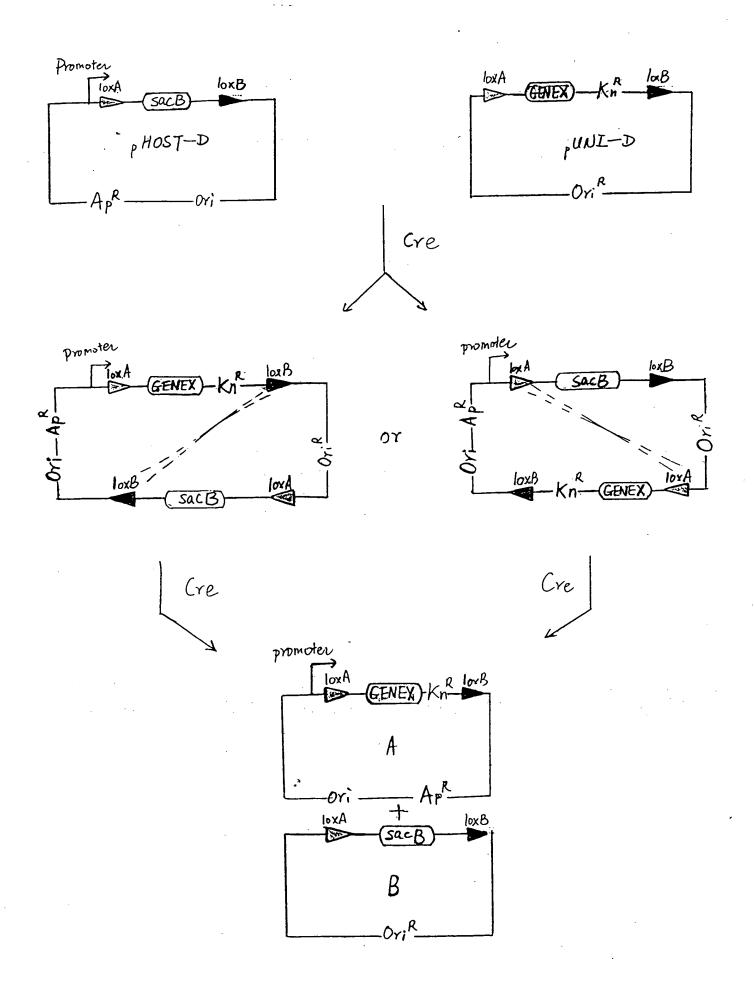
B



A



B



loxP: ATAACTTCGTATA GCATACAT TATACGAAGTTAT
1 2 3 4 5 6 7 8 910111213 13121110 9 8 7 6 5 4 3 2 1

13121110 9 8 7 6 5 4 3 2 1

1 2 3 4 5 6 7 8 910111213

loxP3: ATAACTTCGTATA GCATACAT TATA CGAAGTTAT
1 2 3 4 5 6 7 8 910111213 13121110 9 8 7 6 5 4 3 2 1

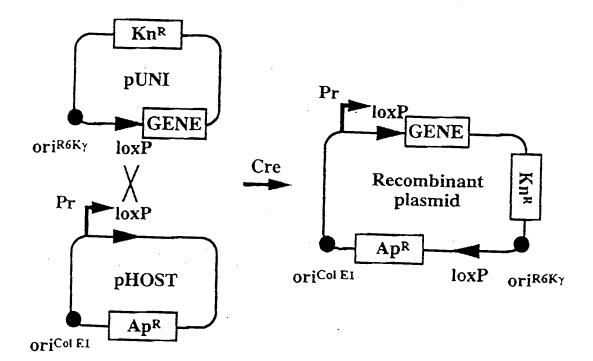
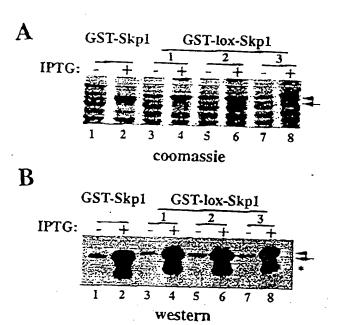
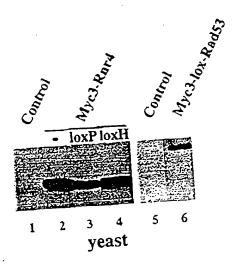


FIGURE 15

GST-Cre (μg)	number of Ap ^R transformants	number of Kn ^R transformants	Kn ^R /Ap ^R (%)
0	4.0 x 10 ⁵	0	0
0.02	3.0 x 10 ⁵	231	0.1
0.04	2.3×10^{5}	406	0.2
0.06	2.4×10^{5}	868	0.4
0.08	3.3 x 10 ⁵	1,336	0.4
0.10	6.0×10^{4}	594	1.0
0.20	7.8 x 10 ⁴	580	0.7
0.40	5.8 x 10 ⁴	1,910	3.3
0.60	9.2 x 10⁴	10,750	11.7
0.80	3.1 x 10 ⁵	28,660	9.2
1.00	1.0×10^{5}	16,840	16.8

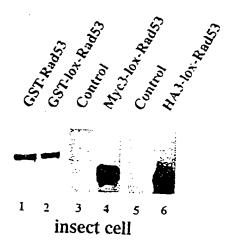




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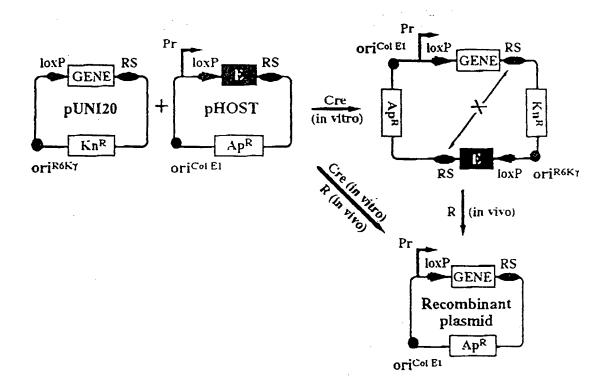
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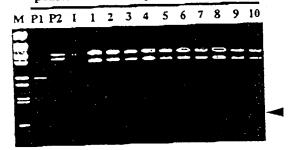
Control HAS. lor. PS.

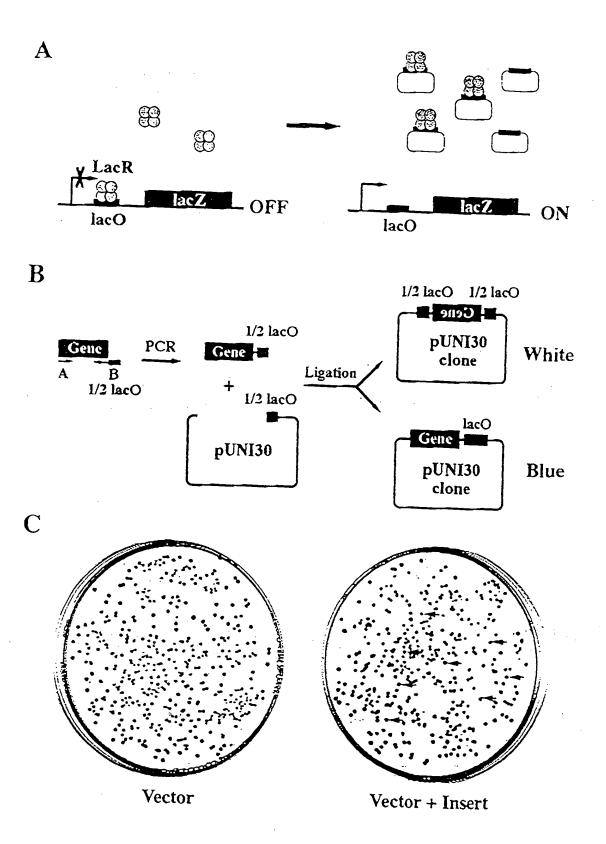
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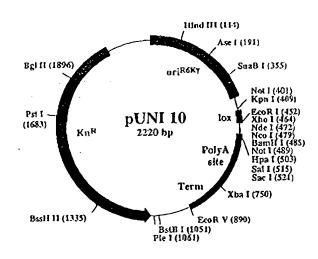
mammalian

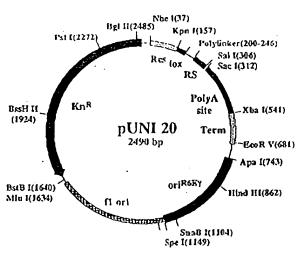


Pvu II restriction analysis of recombinant plasmids made by one-step POT









pUNI 10 Polylinker Sequence

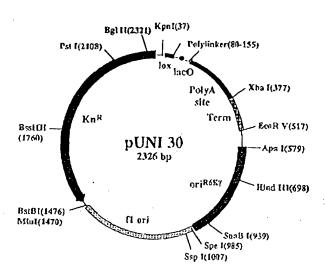
pUNI 20 Polylinker Sequence

(401) Not 1	Kpal	LOX							
CC CCC CC	CGGTACCA	TA ACT T	CG TAT AC	C ATA CAT	TAT ACG A				
AG TTA TCT	EmR1	Sma I	XIIO I	Nde I	Nco I				
BamHI GG ATC CG	Not I	H ATTÖTT	AA CAG A	Sal 1	Sac I (S30)				

(157) Kpnl LOX
GGT ACC ATA ACT TCG TAT AGC ATA CAT TAT ACG AAG TTA T

EcoR I Small Xholl Ndel Ncol Banill CT GGA ATT CCC CGG GCT CGA GAACAT ATG GCC ATG GGG ATC

Not 1 (246) CGC GGC CGC

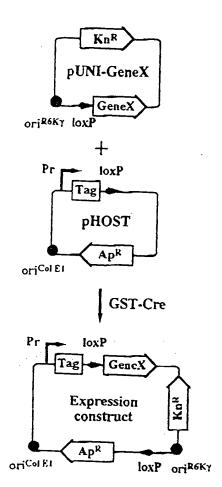


pUNI 30 Polylinker Sequence

GGT ACC ATA ACT TCG TAT AGC ATA CAT TAT ACG AAG TTA TC

EcoR I Sma I Xho I Ecotili
T GGA ATT CCC CGG GCT CGA GCC AGT CCA GCG CTC ACA ATT
half IacO

Not 1 II pal Sat Sact (155)
GCG GCC GCA ATT GTT AAC AGA TCC GTC GAC GAG CTC GC
Muni



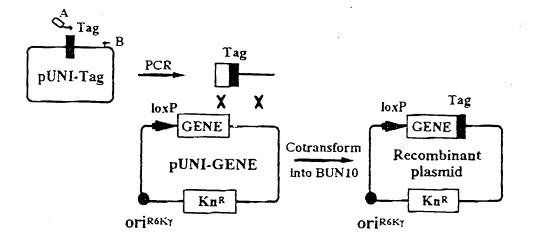


FIGURE 26A

SEQUENCE DESCRIPTION:	SEQ ID NO:1:							
AATTCTGTCA GCCGTTAAGT	GTTCCTGTGT	CACTGAAAAT	TGCTTTGAGA	GGCTCTAAGG				
GCTTCTCAGT GCGTTACATC	CCTGGCTTGT	TGTCCACAAC	CGTTAAACCT	TAAAAGCTTT				
AAAAGCCTTA TATATTCTTT 180	TTTTTCTTAT	AAAACTTAAA	ACCTTAGAGG	CTATTTAAGT				
TGCTGATTTA TATTAATTTT 240	ATTGTTCAAA	CATGAGAGCT	TAGTACGTGA	AACATGAGAG				
CTTAGTACGT TAGCCATGAG 300	AGCTTAGTAC	GTTAGCCATG	AGGGTTTAGT	TCGTTAAACA				
TGAGAGCTTA GTACGTTAAA 360	CATGAGAGCT	TAGTACGTGA	AACATGAGAG	CTTAGTACGT				
ACTATCAACA GGTTGAACTG 420	CTGATCAACA	GATCCTCTAC	GCGGCCGCGG	TACCATAACT				
TCGTATAGCA TACATTATAC 480	GAAGTTATCT	GGAATTCCCC	GGGCTCGAGA	ACATATGGCC				
ATGGGGATCC GCGGCCGCAA 540	TTGTTAACAG	ATCCGTCGAC	GAGCTCGCTA	TCAGCCTCGA				
CTGTGCCTTC TAGTTGCCAG	CCATCTGTTG	TTTGCCCCTC	CCCCGTGCCT	TCCTTGACCC				
TGGAAGGTGC CACTCCCACT	GTCCTTTCCT	AATAAAATGA	GGAAATTGCA	TCGCATTGTC				
TGAGTAGGTG TCATTCTATT 720	CTGGGGGGTG	GGGTGGGGCA	GGACAGCAAG	GGGGAGGATT				
GGGAAGACAA TAGCAGGCAT 780	GCTGGGGATT	CTAGAAGATC	CGGCTGCTAA	CAAAGCCCGA				
AAGGAAGCTG AGTTGGCTGC 840	TGCCACCGCT	GAGCAATAAC	TAGCATAACC	CCTTGGGGCC				
TCTAAACGGG TCTTGAGGGG 900	TTTTTTGCTG	AAAGGAGGAA	CTATATCCGG	ATATCCCGGG				
GTGGGCGAAG AACTCCAGCA 960	TGAGATCCCC	GCGCTGGAGG	ATCATCCAGC	CGGCGTCCCG				
GAAAACGATT CCGAAGCCCA 1020	ACCTTTCATA	GAAGGCGGCG	GTGGAATCGA	AATCTCGTGA				
TGGCAGGTTG GGCGTCGCTT	GGTCGGTCAT	TTCGAACCCC	AGAGTCCCGC	TCAGAAGAAC				

- TCGTCAAGAA GGCGATAGAA GGCGATGCGC TGCGAATCGG GAGCGGCGAT ACCGTAAAGC 1140
 - ACGAGGAAGC GGTCAGCCCA TTCGCCGCCA AGCTCTTCAG CAATATCACG GGTAGCCAAC 1200
 - GCTATGTCCT GATAGCGGTC CGCCACACCC AGCCGGCCAC AGTCGATGAA TCCAGAAAAG 1260
 - CGGCCATTTT CCACCATGAT ATTCGGCAAG CAGGCATCGC CATGGGTCAC GACGAGATCC 1320
 - TCGCCGTCGG GCATGCGCGC CTTGAGCCTG GCGAACAGTT CGGCTGGCGC GAGCCCCTGA
 - TGCTCTTCGT CCAGATCATC CTGATCGACA AGACCGGCTT CCATCCGAGT ACGTGCTCGC 1440
 - TCGATGCGAT GTTTCGCTTG GTGGTCGAAT GGGCAGGTAG CCGGATCAAG CGTATGCAGC 1500
 - CGCCGCATTG CATCAGCCAT GATGGATACT TTCTCGGCAG GAGCAAGGTG AGATGACAGG
 - AGATCCTGCC CCGGCACTTC GCCCAATAGC AGCCAGTCCC TTCCCGCTTC AGTGACAACG 1620
 - TCGAGCACAG CTGCGCAAGG AACGCCCGTC GTGGCCAGCC ACGATAGCCG CGCTGCCTCG 1680
 - TCCTGCAGTT CATTCAGGGC ACCGGACAGG TCGGTCTTGA CAAAAAGAAC CGGGCGCCCC 1740
 - TGCGCTGACA GCCGGAACAC GGCGGCATCA GAGCAGCCGA TTGTCTGTTG TGCCCAGTCA 1800
 - TAGCCGAATA GCCTCTCCAC CCAAGCGGCC GGAGAACCTG CGTGCAATCC ATCTTGTTCA 1860
 - ATCATGCGAA ACGATCCTCA TCCTGTCTCT TGATCAGATC TTGATCCCCT GCGCCATCAG
 - ATCCTTGGCG GCAAGAAAGC CATCCAGTTT ACTTTGCAGG GCTTCCCAAC CTTACCAGAG 1980
 - GGCGCCCCAG CTGGCAATTC CGGTTCGCTT GCTGTCCATA AAACCGCCCA GTCTAGCTAT 2040
 - CGCCATGTAA GCCCACTGCA AGCTACCTGC TTTCTCTTTG CGCTTGCGTT TTCCCTTGTC 2100

CAGATAGCCC AGTAGCTGAC ATTCATCCGG GGTCAGCACC GTTTCTGCGG ACTGGCTTTC 2160

TACGTGTTCC GCTTCCTTTA GCAGCCCTTG CGCCCTGAGT GCTTGCGGCA GCGTGAAGCT 2220

FIGURE 26B

SEQUENCE DESCRIPTION: SEQ ID NO:10:

ATG 48	TCC	CCT	ATA	CTA	GGT	TAT	TGG	AAA	ATT	AAG	GGC	CTT	GTG	CAA	CCC
	Ser	Pro	Ile	Leu 5	Gly	Tyr	Trp	Lys	Ile 10	Lys	Gly	Leu	Val	Gln 15	Pro
ACT 96	CGA	CTT	CTT	TTG	GAA	TAT	CTT	GAA	GAA	AAA	TAT	GAA	GAG	CAT	TTG
-	Arg	Leu	Leu 20	Leu	Glu	Tyr	Leu	Glu 25	Glu	Lys	Tyr	Glu	Glu 30	His	Leu
TAT 144	GAG	CGC	GAT	GAA	GGT	GAT	AAA	TGG	CGA	AAC	AAA	AAG	TTT	GAA	TTG
	Glu	Arg 35	Asp	Glu	Gly	Asp	Lys 40	Trp	Arg	Asn	Lys	Lys 45	Phe	Glu	Leu
GGT 192	TTG	GAG	TTT	CCC	AAT	CTT	CCT	TAT	TAT	ATT	GAT	GGT	GAT	GTT	AAA
	Leu 50	Glu	Phe	Pro	Asn	Leu 55	Pro	Tyr	Tyr	Ile	Asp 60	Gly	Asp	Val	Lys
TTA 240	ACA	CAG	TCT	ATG	GCC	ATC	ATA	CGT	TAT	ATA	GCT	GAC	AAG	CAC	AAC
	Thr	Gln	Ser	Met	Ala 70	Ile	Ile	Arg	Tyr	Ile 75	Ala	Asp	Lys	His	Asn 80
ATG 288	TTG	GGT	GGT	TGT	ĊCA	AAA	GAG	CGT	GCA	GAG	ATT	TCA	ATG	CTT	GAA
	Leu	Gly	Gly	Cys 85	Pro	Lys	Glu	Arg	Ala 90	Glu	Ile	Ser	Met	Leu 95	Glu
GGA 336	GCG	GTT	TTG	GAT	ATT	AGA	TAC	GGT	GTT	TCG	AGA	ATT	GCA	TAT	AGT
	Ala	Val	Leu 100	Asp	Ile	Arg	Tyr	Gly 105	Val	Ser	Arg	Ile	Ala 110	Tyr	Ser
AAA 384	GAC	TTT	GAA	ACT	CTC	AAA	GTT	GAT	TTT	CTT	AGC	AAG	CTA	CCT	GAA
Lys	Asp	Phe 115	Glu	Thr	Leu	Lys	Val 120	Asp	Phe	Leu	Ser	Lys 125	Leu	Pro	Glu
ATG 432		AAA	ATG	TTC	GAA	GAT	CGT	TTA	TGT	CAT	AAA	ACA	TAT	TTA	AAT
		Lys	Met	Phe	Glu	Asp 135	Arg	Leu	Cys	His	Lys 140	Thr	Tyr	Leu	Asn
GGT 480	GAT	CAT	GTA	ACC	CAT	CCT	GAC	TTC	ATG	TTG	TAT	GAC	GCT	CTT	GAT
	Asp	His	Val	Thr	His 150	Pro	Asp	Phe	Met	Leu 155	Tyr	Asp	Ala	Leu	Asp 160

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GTT GTT TTA TAC ATG GAC CCA ATG TGC CTG GAT GCG TTC CCA AAA TTA
528
Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro Lys Leu
GTT TGT TTT AAA AAA CGT ATT GAA GCT ATC CCA CAA ATT GAT AAG TAC
Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro Gln Ile Asp Lys Tyr
                                185
TTG AAA TCC AGC AAG TAT ATA GCA TGG CCT TTG CAG GGC TGG CAA GCC
624
Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp Gln Ala
ACG TTT GGT GGC GAC CAT CCT CCA AAA TCG GAT CTG GTT CCG CGT
Thr Phe Gly Gly Asp His Pro Pro Lys Ser Asp Leu Val Pro Arg
                        215
    210
                                            220
GGA TCT CGT CGT GCA TCT GTT GGA TCG CAT ATG CCC ATG GCC AAT TTA
Gly Ser Arg Arg Ala Ser Val Gly Ser His Met Pro Met Ala Asn Leu
CTG ACC GTA CAC CAA AAT TTG CCT GCA TTA CCG GTC GAT GCA ACG AGT
Leu Thr Val His Gln Asn Leu Pro Ala Leu Pro Val Asp Ala Thr Ser
                245
                                    250
                                                        255
GAT GAG GTT CGC AAG AAC CTG ATG GAC ATG TTC AGG GAT CGC CAG GCG
Asp Glu Val Arg Lys Asn Leu Met Asp Met Phe Arg Asp Arg Gln Ala
TTT TCT GAG CAT ACC TGG AAA ATG CTT CTG TCC GTT TGC CGG TCG TGG
864
Phe Ser Glu His Thr Trp Lys Met Leu Leu Ser Val Cys Arq Ser Trp
        275
GCG GCA TGG TGC AAG TTG AAT AAC ∕CGG AAA TGG TTT CCC GCA GAA CCT
Ala Ala Trp Cys Lys Leu Asn Asn Arg Lys Trp Phe Pro Ala Glu Pro
    290
                        295
GAA GAT GTT CGC GAT TAT CTT CTA TAT CTT CAG GCG CGC GGT CTG GCA
Glu Asp Val Arg Asp Tyr Leu Leu Tyr Leu Gln Ala Arg Gly Leu Ala
GTA AAA ACT ATC CAG CAA CAT TTG GGC CAG CTA AAC ATG CTT CAT CGT
1008
Val Lys Thr Ile Gln Gln His Leu Gly Gln Leu Asn Met Leu His Arg
                325
                                    330
                                                        335
CGG TCC GGG CTG CCA CGA CCA AGT GAC AGC AAT GCT GTT TCA CTG GTT
Arg Ser Gly Leu Pro Arg Pro Ser Asp Ser Asn Ala Val Ser Leu Val
            340
                                345
                                                    350
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ATG CGG CGG ATC CGA AAA GAA AAC GTT GAT GCC GGT GAA CGT GCA AAA 1104 Met Arg Arg Ile Arg Lys Glu Asn Val Asp Ala Gly Glu Arg Ala Lys 355 CAG GCT CTA GCG TTC GAA CGC ACT GAT TTC GAC CAG GTT CGT TCA CTC 1152 Gln Ala Leu Ala Phe Glu Arg Thr Asp Phe Asp Gln Val Arg Ser Leu ATG GAA AAT AGC GAT CGC TGC CAG GAT ATA CGT AAT CTG GCA TTT CTG Met Glu Asn Ser Asp Arg Cys Gln Asp Ile Arg Asn Leu Ala Phe Leu 390 395 GGG ATT GCT TAT AAC ACC CTG TTA CGT ATA GCC GAA ATT GCC AGG ATC 1248 Gly Ile Ala Tyr Asn Thr Leu Leu Arg Ile Ala Glu Ile Ala Arg Ile 405 410 415 AGG GTT AAA GAT ATC TCA CGT ACT GAC GGT GGG AGA ATG TTA ATC CAT 1296 Arg Val Lys Asp Ile Ser Arg Thr Asp Gly Gly Arg Met Leu Ile His 420 425 ATT GGC AGA ACG AAA ACG CTG GTT AGC ACC GCA GGT GTA GAG AAG GCA 1344 Ile Gly Arg Thr Lys Thr Leu Val Ser Thr Ala Gly Val Glu Lys Ala 435 445 CTT AGC CTG GGG GTA ACT AAA CTG GTC GAG CGA TGG ATT TCC GTC TCT 1392 Leu Ser Leu Gly Val Thr Lys Leu Val Glu Arg Trp Ile Ser Val Ser 460 GGT GTA GCT GAT GAT CCG AAT AAC TAC CTG TTT TGC CGG GTC AGA AAA Gly Val Ala Asp Asp Pro Asn Asn Tyr Leu Phe Cys Arg Val Arg Lys 465 470 475 480 AAT GGT GTT GCC GCG CCA TCT GCC ACC AGC CAG CTA TCA ACT CGC GCC 1488 Asn Gly Val Ala Ala Pro Ser Ala Thr Ser Gln Leu Ser Thr Arg Ala 485 490 CTG GAA GGG ATT TTT GAA GCA ACT CAT CGA TTG ATT TAC GGC GCT AAG 1536 Leu Glu Gly Ile Phe Glu Ala Thr His Arg Leu Ile Tyr Gly Ala Lys 500 GAT GAC TCT GGT CAG AGA TAC CTG GCC TGG TCT GGA CAC AGT GCC CGT 1584 Asp Asp Ser Gly Gln Arg Tyr Leu Ala Trp Ser Gly His Ser Ala Arg 515 GTC GGA GCC GCG CGA GAT ATG GCC CGC GCT GGA GTT TCA ATA CCG GAG 1632 Val Gly Ala Ala Arg Asp Met Ala Arg Ala Gly Val Ser Ile Pro Glu 530 535 540

ATC ATG CAA GCT GGT GGC TGG ACC AAT GTA AAT ATT GTC ATG AAC TAT 1680

Ile Met Gln Ala Gly Gly Trp Thr Asn Val Asn Ile Val Met Asn Tyr 545 550 550 560

ATC CGT AAC CTG GAT AGT GAA ACA GGG GCA ATG GTG CGC CTG CTG GAA 1728

Ile Arg Asn Leu Asp Ser Glu Thr Gly Ala Met Val Arg Leu Leu Glu
565 570 575

GAT GGC GAT TAG 1740 Asp Gly Asp

FIGURE 26C

SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Ser Pro Ile Leu Gly Tyr Trp Lys Ile Lys Gly Leu Val Gln Pro Thr Arg Leu Leu Glu Tyr Leu Glu Glu Lys Tyr Glu Glu His Leu Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe Glu Leu Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp Val Lys Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys His Asn Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met Leu Glu Gly Ala Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala Tyr Ser Lys Asp Phe Glu Thr Leu Lys Val Asp Phe Leu Ser Lys Leu Pro Glu 120 125 Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys Thr Tyr Leu Asn 130 Gly Asp His Val Thr His Pro Asp Phe Met Leu Tyr Asp Ala Leu Asp 150 155 Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro Lys Leu Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro Gln Ile Asp Lys Tyr 185 Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp Gln Ala Thr Phe Gly Gly Gly Asp His Pro Pro Lys Ser Asp Leu Val Pro Arg Gly Ser Arg Arg Ala Ser Val Gly Ser His Met Pro Met Ala Asn Leu 230 235 Leu Thr Val His Gln Asn Leu Pro Ala Leu Pro Val Asp Ala Thr Ser 250 Asp Glu Val Arg Lys Asn Leu Met Asp Met Phe Arg Asp Arg Gln Ala 265 260 270 Phe Ser Glu His Thr Trp Lys Met Leu Leu Ser Val Cys Arg Ser Trp

285

Ala Ala Trp Cys Lys Leu Asn Asn Arg Lys Trp Phe Pro Ala Glu Pro 290 295 300

Glu Asp Val Arg Asp Tyr Leu Leu Tyr Leu Gln Ala Arg Gly Leu Ala 305 310 315 320

Val Lys Thr Ile Gln Gln His Leu Gly Gln Leu Asn Met Leu His Arg 325 330 335

Arg Ser Gly Leu Pro Arg Pro Ser Asp Ser Asn Ala Val Ser Leu Val 340 345 350

Met Arg Arg Ile Arg Lys Glu Asn Val Asp Ala Gly Glu Arg Ala Lys 355 360 365

Gln Ala Leu Ala Phe Glu Arg Thr Asp Phe Asp Gln Val Arg Ser Leu 370 375 380

Met Glu Asn Ser Asp Arg Cys Gln Asp Ile Arg Asn Leu Ala Phe Leu 385 390 395 400

Gly Ile Ala Tyr Asn Thr Leu Leu Arg Ile Ala Glu Ile Ala Arg Ile
405 410 415

Arg Val Lys Asp Ile Ser Arg Thr Asp Gly Gly Arg Met Leu Ile His 420 425 430

Ile Gly Arg Thr Lys Thr Leu Val Ser Thr Ala Gly Val Glu Lys Ala
435 440 445

Leu Ser Leu Gly Val Thr Lys Leu Val Glu Arg Trp Ile Ser Val Ser 450 455 460

Gly Val Ala Asp Asp Pro Asn Asn Tyr Leu Phe Cys Arg Val Arg Lys
465 470 475 480

Asn Gly Val Ala Ala Pro Ser Ala Thr Ser Gln Leu Ser Thr Arg Ala
485 490 495

Leu Glu Gly Ile Phe Glu Ala Thr His Arg Leu Ile Tyr Gly Ala Lys 500 505 510

Asp Asp Ser Gly Gln Arg Tyr Leu Ala Trp Ser Gly His Ser Ala Arg 515 520 525

Val Gly Ala Ala Arg Asp Met Ala Arg Ala Gly Val Ser Ile Pro Glu
530 540

Ile Met Gln Ala Gly Gly Trp Thr Asn Val Asn Ile Val Met Asn Tyr 545 550 560

Ile Arg Asn Leu Asp Ser Glu Thr Gly Ala Met Val Arg Leu Leu Glu
565 570 575

Asp Gly Asp